Available online at www.sciencedirect.com
SCIENCE DIRECT
Renewable and Sustainable Energy Reviews
10 (2006) 248–268
RENEWABLE & SUSTAINABLE ENERGY REVIEWS
www.elsevier.com/locate/rser

Technical aspects of biodiesel production by transesterification—a review

L.C. Meher, D. Vidya Sagar, S.N. Naik*

Centre for Rural Development and Technology, Indian Institute of Technology Delhi, New Delhi 110016, India

Received 17 February 2004; accepted 15 September 2004

Abstract

Biodiesel is gaining more and more importance as an attractive fuel due to the depleting fossil fuel resources. Chemically biodiesel is monoalkyl esters of long chain fatty acids derived from renewable feed stock like vegetable oils and animal fats. It is produced by transesterification in which, oil or fat is reacted with a monohydric alcohol in presence of a catalyst. The process of transesterification is affected by the mode of reaction condition, molar ratio of alcohol to oil, type of alcohol, type and amount of catalysts, reaction time and temperature and purity of reactants. In the present paper various methods of preparation of biodiesel with different combination of oil and catalysts have been described. The technical tools and processes for monitoring the transesterification reactions like TLC, GC, HPLC, GPC, ^{1}H NMR and NIR have also been summarized. In addition, fuel properties and specifications provided by different countries are discussed.

Keywords: Transesterification; Vegetable oil; Biodiesel; Fatty acid alkyl esters; Reaction condition; Analytical methods

Contents

* Corresponding author. Tel.: +91 11 26591162; fax: +91 11 26591121.
E-mail address: snn@rdat.iitd.ernet.in (S.N. Naik).

1364-0321/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.rser.2004.09.002

1. Introduction

1.1. Background

Majority of the worlds energy needs are supplied through petrochemical sources, coal and natural gases, with the exception of hydroelectricity and nuclear energy, of all, these sources are finite and at current usage rates will be consumed shortly [1]. Diesel fuels have an essential function in the industrial economy of a developing country and used for transport of industrial and agricultural goods and operation of diesel tractor and pump sets in agricultural sector. Economic growth is always accompanied by commensurate increase in the transport. The high energy demand in the industrialized world as well as in the domestic sector, and pollution problems caused due to the widespread use of fossil fuels make it increasingly necessary to develop the renewable energy sources of limitless duration and smaller environmental impact than the traditional one. This has stimulated recent interest in alternative sources for petroleum-based fuels. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available. One possible alternative to fossil fuel is the use of oils of plant origin like vegetable oils and tree borne oil seeds. This alternative diesel fuel can be termed as

biodiesel. This fuel is biodegradable and non-toxic and has low emission profiles as compared to petroleum diesel. Usage of biodiesel will allow a balance to be sought between agriculture, economic development and the environment.

Chemically the oils/fats consist of triglyceride molecules of three long chain fatty acids that are ester bonded to a single glycerol molecule. These fatty acids differ by the length of carbon chains, the number, orientation and position of double bonds in these chains. Thus biodiesel refers to lower alkyl esters of long chain fatty acids, which are synthesized either by transesterification with lower alcohols or by esterification of fatty acids.

1.2. Vegetable oil as fuel

The use of vegetable oils as alternative fuels has been around for 100 years when the inventor of the diesel engine Rudolph Diesel first tested peanut oil, in his compression ignition engine [2]. He said, "The use of vegetable oils for engine fuels may seem insignificant today. But such oils may in course of time be as important as petroleum and the coal tar products of the present time". However, due to cheap petroleum products, such non-conventional fuels never took off.

Vegetable oils occupy a prominent position in the development of alternative fuels although, there have been many problems associated with using it directly in diesel engine (especially in direct injection engine). These include;

1. Coking and trumpet formation on the injectors to such an extent that fuel atomization does not occur properly or even prevented as a result of plugged orifices,
2. Carbon deposits,
3. Oil ring sticking,
4. Thickening or gelling of the lubricating oil as a result of contamination by vegetable oils, and
5. Lubricating problems.

Other disadvantages to the use of vegetable oils and especially animal fats are the high viscosity (about 11–17 times higher than diesel fuel), lower volatilities that causes the formation of deposits in engines due to incomplete combustion and incorrect vaporization characteristics. These problems are associated with large triglyceride molecule and its higher molecular mass and avoided by modifying the engine less or more according to the conditions of use and the oil involved. The modified engines built by Elsbett in Germany and Malaysia and Diesel Morten und Geraetebau GmbH (DMS) in Germany and in USA show a good performance when fuelled with vegetable oils of different composition and grades [1].

1.3. Biodiesel

The plant oils usually contain free fatty acids, phospholipids, sterols, water, odourants and other impurities. Because of these, the oil cannot be used as fuel directly. To overcome these problems the oil requires slight chemical modification mainly transesterification,

pyrolysis and emulsification. Among these, the transesterification is the key and foremost important step to produce the cleaner and environmentally safe fuel from vegetable oils.

Biodiesel is the monoalkyl esters of long chain fatty acids derived from renewable feed stocks, such as vegetable oil or animal fats, for use in compression ignition engine. Biodiesel, which is considered as a possible substitute of conventional diesel fuel is commonly, composed of fatty acid methyl esters that can be prepared from triglycerides in vegetable oils by transesterification with methanol. The resulting biodiesel is quite similar to conventional diesel fuel in its main characteristics.

2. Transesterification

Transesterification or alcoholysis is the displacement of alcohol from an ester by another in a process similar to hydrolysis, except than alcohol is used instead of water [1]. This process has been widely used to reduce the high viscosity of triglycerides. The transesterification reaction is represented by the general equation as Fig. 1.

If methanol is used in this process it is called methanolysis. Methanolysis of triglyceride is represented in Fig. 2.

Transesterification is one of the reversible reactions and proceeds essentially by mixing the reactants. However, the presence of a catalyst (a strong acid or base) accelerates the conversion.

3. Transesterifcation kinetics and mechanism

Transesterification of triglycerides produce fatty acid alkyl esters and glycerol. The glycerol layer settles down at the bottom of the reaction vessel. Diglycerides and monoglycerides are the intermediates in this process. The mechanism of transesterification is described in Fig. 3.

The step wise reactions are reversible and a little excess of alcohol is used to shift the equilibrium towards the formation of esters. In presence of excess alcohol, the foreword reaction is pseudo-first order and the reverse reaction is found to be second order. It was also observed that transesterification is faster when catalyzed by alkali [3].

The mechanism of alkali-catalyzed transesterification is described in Fig. 4. The first step involves the attack of the alkoxide ion to the carbonyl carbon of the triglyceride molecule, which results in the formation of a tetrahedral intermediate. The reaction of this intermediate with an alcohol produces the alkoxide ion in the second step. In the last step the rearrangement of the tetrahedral intermediate gives rise to an ester and a diglyceride [4].

$$\underset{\text{Ester}}{RCOOR^1} + \underset{\text{Alcohol}}{R^2OH} \xrightleftharpoons{\text{Catalyst}} \underset{\text{Ester}}{RCOOR^2} + \underset{\text{Alcohol}}{R^1OH}$$

Fig. 1. General equation of transesterification.

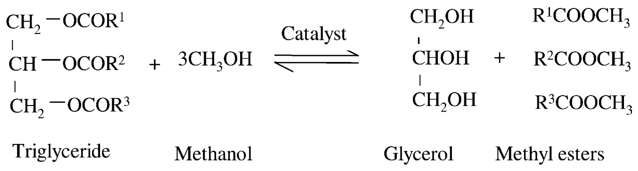


Fig. 2. General equation for transesterification of triglycerides.

Transesterification can be catalyzed by Brownsted acids, preferably by sulfonic and sulfuric acids. These catalysts give very high yields in alkyl esters but these reactions are slow, requiring typically temperature above 100 °C and more than 3 h to complete the conversion [5]. The mechanism of acid catalyzed transesterification of vegetable oil (for a monoglyceride) is shown in Fig. 5. However, it can be extended to di- and tri-glycerides. The protonation of carbonyl group of the ester leads to the carbocation, which after a nucleophilic attack of the alcohol produces a tetrahedral intermediate. This intermediate eliminates glycerol to form a new ester and to regenerate the catalyst.

The methanolysis of soybean oil in the presence of 1% H_2SO_4 with an alcohol/oil molar ratio 30:1 was studied. At a reaction temperature of 65 °C the conversion was observed to be completed in 20 h, while butanolysis at 117 °C and ethanolysis at 78 °C using the same quantities of catalyst and alcohol, take 3 and 18 h, respectively [3].

4. Variables affecting transesterification reaction

The process of transesterification is affected by various factors depending upon the reaction condition used. The effects of these factors are described below.

4.1. Effect of free fatty acid and moisture

The free fatty acid and moisture content are key parameters for determining the viability of the vegetable oil transesterification process. To carry the base catalyzed reaction to completion; an free fatty acid (FFA) value lower than 3% is needed. The higher the acidity of the oil, smaller is the conversion efficiency. Both, excess as well as insufficient amount of catalyst may cause soap formation [6].

Ma et al. [7] studied the transesterification of beef tallow catalyzed by NaOH in presence of free fatty acids and water. Without adding FFA and water, the apparent yield of beef tallow methyl esters (BTME) was highest. When 0.6% of FFA was added, the apparent yield of BTME reached the lowest, less than 5%, with any level of water added.

$$\text{Triglyceride} + R^1OH \rightleftharpoons \text{Diglyceride} + RCOOR^1$$

$$\text{Diglyceride} + R^1OH \rightleftharpoons \text{Monoglyceride} + RCOOR^1$$

$$\text{Monoglyceride} + R^1OH \rightleftharpoons \text{Glycerol} + RCOOR^1$$

Fig. 3. General equation for transesterification of triglycerides.

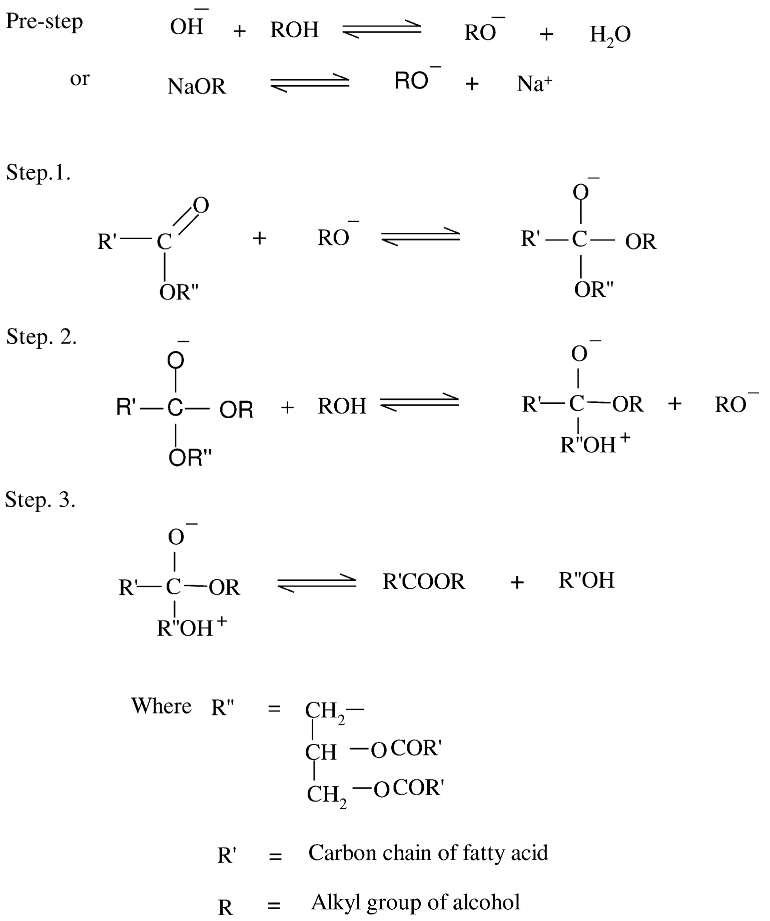


Fig. 4. Mechanism of base catalyzed transesterification.

The products were solid at room temperature, similar to the original beef tallow. When 0.9% of water was added, without addition of FFA, the apparent yield was about 17%. If the low qualities of beef tallow or vegetable oil with high FFA are used to make biodiesel fuel, they must be refined by saponification using NaOH solution to remove free fatty acids. Conversely, the acid catalyzed process can also be used for esterification of these free fatty acids.

The starting materials used for base catalyzed alcoholysis should meet certain specifications. The triglycerides should have lower acid value and all material should be substantially anhydrous. The addition of more sodium hydroxide catalyst compensates for higher acidity, but the resulting soap causes an increase in viscosity or formation of gels that interferes in the reaction as well as with separation of glycerol [8]. When the reaction conditions do not meet the above requirements, ester yields are significantly reduced. The methoxide and hydroxide of sodium or potassium should be maintained in anhydrous

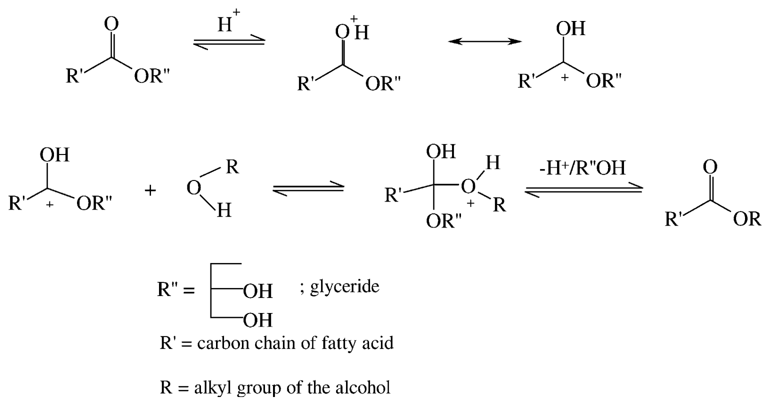


Fig. 5. Mechanism of acid catalyzed transesterification.

state. Prolonged contact with air will diminish the effectiveness of these catalysts through interaction with moisture and carbon dioxide.

Most of the biodiesel is currently made from edible oils by using methanol and alkaline catalyst. However, there are large amounts of low cost oils and fats that could be converted to biodiesel. The problems with processing these low cost oils and fats is that they often contain large amounts of free fatty acids that cannot be converted to biodiesel using alkaline catalyst. Therefore, two-step esterification process is required for these feed stocks. Initially the FFA of these can be converted to fatty acid methyl esters by an acid catalyzed pretreatment and in the second step transesterification is completed by using alkaline catalyst to complete the reaction [9]. Initial process development was performed with synthetic mixture containing 20 and 40% free fatty acid prepared by using palmitic acid. Process parameters such as molar ratio of alcohol to oil, type of alcohol, amount of acid catalyst, reaction time, free fatty acid level were investigated to determine the best strategy for converting the free fatty acids to usable esters. The work showed that the acid level of the high free fatty acids feed stocks could be reduced to less than 1% with a two-step pretreatment reaction. The reaction mixture was allowed to settle between steps so that the water containing phase could be removed. The two-step pretreatment reaction was demonstrated with actual feedstocks, including yellow grease with 12% free fatty acid and brown grease with 33% free fatty acids. After reducing the acid levels of these feedstocks to less than 1%, the transesterification reaction was completed with an alkaline catalyst to produce fuel grade biodiesel.

Turck et al. [10] have investigated the negative influence of base catalyzed transesterification of triglycerides containing substantial amount of free fatty acid. Free fatty acids react with the basic catalyst added for the reaction and give rise to soap, as a result of which, one part of the catalyst is neutralized and is therefore no longer available for transesterification. These high FFA content oils/fats are processed with an immiscible basic glycerol phase so as to neutralize the free fatty acids and cause them to pass over into the glycerol phase by means of monovalent alcohols. The triglycerides are subjected to transesterification, using a base as catalyst, to form fatty acid alkyl esters, characterized in that after its separation, the basic glycerol phase produced during transesterification of

the triglycerides is used for processing the oils/fats for removal of free fatty acids. The minimum amount of catalyst required for this process was calculated, relative to 1000 g of the oil to be processed, as a function of the acid value and the mean molar mass of the oil/fat.

4.2. Catalyst type and concentration

Catalysts used for the transesterification of triglycerides are classified as alkali, acid, enzyme or heterogeneous catalysts, among which alkali catalysts like sodium hydroxide, sodium methoxide, potassium hydroxide, potassium methoxide are more effective [4]. If the oil has high free fatty acid content and more water, acid catalyzed transesterification is suitable. The acids could be sulfuric acid, phosphoric acid, hydrochloric acid or organic sulfonic acid. Methanolysis of beef tallow was studied with catalysts NaOH and NaOMe. Comparing the two catalysts, NaOH was significantly better than NaOMe [7]. The catalysts NaOH and NaOMe reached their maximum activity at 0.3 and 0.5% w/w of the beef tallow, respectively. Sodium methoxide causes formation of several by-products mainly sodium salts, which are to be treated as waste. In addition, high quality oil is required with this catalyst [11]. This was different from the previous reports [3] in which ester conversion at the 6:1 molar ratio of alcohol/oil for 1% NaOH and 0.5% NaOMe were almost the same after 60 min. Part of the difference may be attributed to the differences in the reaction system used.

As a catalyst in the process of alkaline methanolysis, mostly sodium hydroxide or potassium hydroxide have been used, both in concentration from 0.4 to 2% w/w of oil. Refined and crude oils with 1% either sodium hydroxide or potassium hydroxide catalyst resulted successful conversion. Methanolysis of soybean oil with the catalyst 1% potassium hydroxide has given the best yields and viscosities of the esters [12].

Attempts have been made to use basic alkaline-earth metal compounds in the transesterification of rapeseed oil for production of fatty acid methyl esters. The reaction proceeds if methoxide ions are present in the reaction medium. The alkaline-earth metal hydroxides, alkoxides and oxides catalyzed reaction proceeds slowly as the reaction mixture constitutes a three-phase system oil–methanol-catalyst, which for diffusion reason inhibits the reaction [13]. The catalytic activity of magnesium oxide, calcium hydroxide, calcium oxide, calcium methoxide, barium hydroxide, and for comparison, sodium hydroxide during the transesterification of rapeseed oil was investigated. Sodium hydroxide exhibited the highest catalytic activity in this process. The degree to which the substrates were reacted reached 85% after 30 min of the process and 95% after 1.5 h, which represented a close value to the equilibrium. Barium hydroxide was slightly less active with a conversion of 75% after 30 min. Calcium methoxide was medially active. The degree to which the substrates were reacted was 55% after 30 min. Eighty percents after 1 h and state of reaction equilibrium (93%) was reached after 2.5 h. The rate of reaction was slowest when catalyzed by CaO. Magnesium oxide and calcium hydroxide showed no catalytic activity in rapeseed oil methanolysis.

Acid catalyzed transesterification was studied with waste vegetable oil. The reaction was conducted at four different catalyst concentrations, 0.5, 1.0, 1.5 and 2.25 M HCl in presence of 100% excess alcohol and the result was compared with 2.25 M H_2SO_4

and the decrease in viscosity was observed. H_2SO_4 has superior catalytic activity in the range of 1.5–2.25 M concentration [14].

Although chemical transesterification using an alkaline catalysis process gives high conversion levels of triglycerides to their corresponding methyl esters in short reaction times, the reaction has several drawbacks: it is energy intensive, recovery of glycerol is difficult, the acidic or alkaline catalyst has to be removed from the product, alkaline waste water require treatment, and free fatty acid and water interfere the reaction. Enzymatic catalysts like lipases are able to effectively catalyze the transesterification of triglycerides in either aqueous or non-aqueous systems, which can overcome the problems mentioned above [15]. In particular, the by-products, glycerol can be easily removed without any complex process, and also that free fatty acids contained in waste oils and fats can be completely converted to alkyl esters. On the other hand, in general the production cost of a lipase catalyst is significantly greater than that of an alkaline one.

4.3. Molar ratio of alcohol to oil and type of alcohol

One of the most important variables affecting the yield of ester is the molar ratio of alcohol to triglyceride. The stoichiometric ratio for transesterification requires three moles of alcohol and one mole of triglyceride to yield three moles of fatty acid alkyl esters and one mole of glycerol. However, transesterification is an equilibrium reaction in which a large excess of alcohol is required to drive the reaction to the right. For maximum conversion to the ester, a molar ratio of 6:1 should be used. The molar ratio has no effect on acid, peroxide, saponification and iodine value of methyl esters [12]. However, the high molar ratio of alcohol to vegetable oil interferes with the separation of glycerin because there is an increase in solubility. When glycerin remains in solution, it helps drive the equilibrium to back to the left, lowering the yield of esters. The transesterification of Cynara oil with ethanol was studied at molar ratios between 3:1 and 15:1. The ester yield increased as the molar ratio increased up to a value of 12:1. The best results were for molar ratios between 9:1 and 12:1. For molar ratios less than 6:1, the reaction was incomplete. For a molar ratio of 15:1 the separation of glycerin is difficult and the apparent yield of esters decreased because a part of the glycerol remains in the biodiesel phase. Therefore, molar ratio 9:1 seems to be the most appropriate [16].

The base catalyzed formation of ethyl ester is difficult compared to the formation of methyl esters. Specifically the formation of stable emulsion during ethanolysis is a problem. Methanol and ethanol are not miscible with triglycerides at ambient temperature, and the reaction mixtures are usually mechanically stirred to enhance mass transfer. During the course of reaction, emulsions usually form. In the case of methanolysis, these emulsions quickly and easily break down to form a lower glycerol rich layer and upper methyl ester rich layer. In ethanolysis, these emulsions are more stable and severely complicate the separation and purification of esters [17]. The emulsions are caused in part by formation of the intermediates monoglycerides and diglycerides, which have both polar hydroxyl groups and non-polar hydrocarbon chains. These intermediates are strong-surface active agents. In the process of alcoholysis, the catalyst, either sodium hydroxide or potassium hydroxide is dissolved in polar alcohol phase, in which triglycerides must

transfer in order to react. The reaction is initially mass-transfer controlled and does not conform to expected homogeneous kinetics. When the concentrations of these intermediates reach a critical level, emulsions form. The larger non-polar group in ethanol, relative to methanol, is assumed to be the critical factor in stabilizing the emulsions. However, the concentration of mono- and di-glycerides are very low, then the emulsions become unstable. This emphasizes the necessity for the reaction to be as complete as possible, thereby reducing the concentrations of mono- and di-glycerides.

4.4. Effect of reaction time and temperature

The conversion rate increases with reaction time. Freedman et al. [8] transesterified peanut, cotton-seed, sunflower and soybean oil under the condition of methanol–oil molar ratio 6:1, 0.5% sodium methoxide catalyst and 60 °C. An approximate yield of 80% was observed after 1 min for soybean and sunflower oils. After 1 h, the conversion was almost the same for all four oils (93–98%). Ma et al. [18] studied the effect of reaction time on transesterification of beef tallow with methanol. The reaction was very slow during the first minute due to mixing and dispersion of methanol into beef tallow. From one to 5 min, the reaction proceeds very fast. The production of beef tallow methyl esters reached the maximum value at about 15 min.

Transesterification can occur at different temperatures, depending on the oil used. For the transesterification of refined oil with methanol (6:1) and 1% NaOH, the reaction was studied with three different temperatures [8]. After 0.1 h, ester yields were 94, 87 and 64% for 60, 45 and 32 °C, respectively. After 1 h, ester formation was identical for 60 and 45 °C runs and only slightly lower for the 32 °C run. Temperature clearly influenced the reaction rate and yield of esters [4].

4.5. Mixing intensity

Mixing is very important in the transesterification reaction, as oils or fats are immiscible with sodium hydroxide–methanol solution. Once the two phases are mixed and the reaction is started, stirring is no longer needed. Initially the effect of mixing on transesterification of beef tallow was study by Ma et al. [18]. No reaction was observed without mixing and when NaOH–MeOH was added to the melted beef tallow in the reactor while stirring, stirring speed was insignificant. Reaction time was the controlling factor in determining the yield of methyl esters. This suggested that the stirring speeds investigated exceeded the threshold requirement of mixing.

4.6. Effect of using organic cosolvents

The methoxide base catalyzed methanolysis of soybean oil at 40 °C (methanol–oil molar ratio 6:1) shows that to form methyl esters proceeds approximately more slowly than butanolysis at 30 °C. This is interpreted to be the result of a two phase reaction in which methanolysis occurs only in the methanol phase. Low oil concentration in methanol causes the slow reaction rate; a slow dissolving rate of the oil in methanol causes an initiation period. Intermediate mono- and di-glycerides preferentially remain in

the methanol, and react further, thus explaining the deviation from second order kinetics. The same explanations apply for hydroxide ion catalyzed methanolysis.

In order to conduct the reaction in a single phase, cosolvents like tetrahydrofuran, 1,4-dioxane and diethyl ether were tested. Although, there are other cosolvents, initial study was conducted with tetrahydrofuran. At the 6:1 methanol–oil molar ratio the addition of 1.25 volume of tetrahydrofuran per volume of methanol produces an oil dominant one phase system in which methanolysis speeds up dramatically and occurs as fast as butanolysis. In particular, THF is chosen because its boiling point of 67 °C is only two degrees higher than that of methanol. Therefore at the end of the reaction the unreacted methanol and THF can be co-distilled and recycled [19].

Using tetrahydrofuran, transesterification of soybean oil was carried out with methanol at different concentrations of sodium hydroxide. The ester contents after 1 min for 1.1, 1.3, 1.4 and 2.0% sodium hydroxide were 82.5, 85, 87 and 96.2%, respectively. Results indicated that the hydroxide concentration could be increased up to 1.3 wt%, resulting in 95% methyl ester after 15 min. [20]. Similarly for transesterification of coconut oil using THF/MeOH volume ratio 0.87 with 1% NaOH catalyst, the conversion was 99% in 1 min.

A single-phase process for the esterification of a mixture of fatty acids and triglycerides were investigated. The process comprises forming a single-phase solution of fatty acids and triglyceride in an alcohol selected from methanol and ethanol, the ratio of said alcohol to triglyceride being 15:1–35:1. The solution further comprises a cosolvent in an amount to form the single phase. In a first step, an acid catalyst for the esterification of fatty acid is added. After a period of time, the acid catalyst is neutralized and a base catalyst for the transesterification of triglycerides is added. After a further period of time, esters are separated from the solution [21].

An improved process was investigated for methanolysis and ethanolysis of fatty acid glycerides such as those found in naturally occurring fats and oils derived from plant and animals. The processes comprise solubilizing oil or fat in methanol or ethanol by addition of a cosolvent in order to form a one-phase reaction mixture, and adding an esterification catalyst. The processes proceed quickly, usually in less than 20 min, at ambient temperatures, atmospheric pressure and without agitation. The co-solvent increases the rate of reaction by making the oil soluble in methanol, thus increasing contact of the reactants. The lower alkyl fatty acid monoesters produced by the process can be used as biofuels and are suitable as diesel fuel replacements or additives [22].

5. Transesterification under different conditions

Palm oil was transmethylated continuously at 70 °C in an organic solvent with sodium methoxide as a catalyst. The optimum ratio of toluene to palm oil is 1:1 (v/v). When the methanol to oil molar ratio was 13:1, transmethylation was 96% complete within 60 s. At higher molar ratio (17:1), transmethylation was 99% complete in 15 s. For lower molar ratios of methanol to oil (9:1 and 5.8:1), yields of palm oil methyl esters were 84 and 58%, respectively. Benzene was a good solvent for transmethylation, but the yield of palm oil methyl ester was slightly lower than toluene. Tetrahydrofuran did not accelerate transmethylation [23].

The direct methanolysis of triglycerides in flowing supercritical carbon dioxide by an immobilized lipase is described. The reaction system consists of two syringe pumps for substrate addition and another two syringe pumps for delivering CO_2 at 24.1 MPa. Corn oil was pumped into the carbon dioxide stream at a rate of 4 μL/min and methanol is pumped at 5 μL/min to yield fatty acid methyl ester >98%. Direct methanolysis of soy flakes gives FAME at similar yield. This combined extraction is performed at 17.2 MPa and 50 °C [24].

Transesterification reaction of rapeseed oil in supercritical methanol was investigated without using the catalyst. An experiment has been carried out in the batch-type reaction vessel preheated at 350 and 400 °C and at a pressure of 45–65 MPa, and with a molar ratio of 1:42 of the rapeseed oil to methanol. It was consequently demonstrated that, in a preheating temperature of 350 °C, 450 s of supercritical treatment of methanol was sufficient to convert the rapeseed oil to methyl esters and that, although the prepared methyl esters were basically the same as those of the common method with a basic catalyst, the yield of methyl esters by the former was found to be higher than that by the later. In addition, it was found that this new supercritical methanol process requires the shorter reaction time and simpler purification procedure because of the unused catalyst [25].

Commercially available crude oils and fats contain considerable amounts of free fatty acids. These free fatty acids are known to react with the alkaline catalyst and form saponified products during transesterification reactions for biodiesel fuel production in the conventional commercial process. Purification of the products therefore becomes necessary after the reaction. In addition, it causes a longer production process and increases the production costs. For this aspect, supercritical methanol method without using any catalyst is evaluated in this work for the reaction of free fatty acids. As a result, complete conversion is achieved for saturated fatty acids to methyl esters at temperatures above 400 °C, whereas for unsaturated fatty acids, lower temperature of 350 °C is appropriate, and higher temperature resulted in a degradation of the products. Consequently, a conversion of free fatty acids to methyl esters is highest, over 95%, when treated at 350 °C. Fortunately, this temperature treatment is also most appropriate for transesterification of triglycerides. Thus, the overall conversion process of rapeseed oil to methyl esters is concluded to be adequate at 350 °C. This finding supports the superiority of supercritical methanol process on biodiesel fuel production, compared with the conventional method, in which the production process becomes much simpler and increases the total yield due to methyl esters produced from free fatty acids [26].

6. Analytical monitoring of the reaction

Various analytical methods were developed for analyzing mixtures containing fatty acid esters and mono-, di-, and tri-glycerides obtained by the transesterification of vegetable oils. Analyses were performed by thin layer chromatography/flame ionization detection (TLC/FID) with an Iatroscan TH-10 instrument. Steryl alcohol served as an internal standard. From plots of areas and weight ratios of methyl linoleate, and mono-, di- and tri-linolein, linear equations were developed from which response factors were

calculated. Hydrogen flow rate and developing solvent strongly influenced resolution and base line stability. Variation in scan speed affected compactness of burning and response factors, but not measured composition. A computerized procedure for data analysis were developed so that 30 samples can be completed in 2–3 h. The relative standard deviations were 1–2% for major components in the range of 90–100% range and 6–83% for minor components in the 1–2% range [27].

6.1. Gas chromatographic method

A gas chromatographic method for the simultaneous determination of glycerol, mono-, di-, and tri-glycerides in vegetable oil methyl esters has been developed. Trimethylsilylation of glycerol, mono-, and di-glyderides, followed by GC using a 10 m capillary coated with a 0.1 μm film of DB-5 allows the determination of all analytes in a single GC run.

In principle, glycerol, mono-, di-, and tri-glycerides can be analyzed on highly inert columns coated with apolar stationary phases without derivatization. The inertness of the column, required to obtain good peak shapes and satisfactory recovery, cannot be easily maintained in routine analysis. Trimethylsilylation of the free hydroxyl groups of glycerol, mono- and di-glycerides, however, ensures excellent peak shapes, good recoveries and low detection limits and enormously improves the ruggedness of the procedure. For complete silylation of glycerol and partial glycerides, the conditions of the derivatization reaction have to be controlled carefully. Extensive studies on the silylation of partial glycerides showed that silylation can be obtained under the following conditions; (i) bis-trimethylsilyl trifluoroacetamide (BSTFA) as silylating agent, addition of pyridine or dimethylformamide and heating to 70 °C for 15 min; (ii) BSTFA + 1% trimethylchlor-osilane as silylating agent, addition of pyridine and a reaction time of 15 min at room temperature; (iii) N-methyl N-trimethylsilyl-trifluoroacetamide (MSTFA) as silylating agent, addition of pyridine and reaction time of 15 min at room temperature; (iv) MSTFA as silylating agent and heating to 70 °C for 15 min. The internal standard 1,2,4-butanetriol serves as a very sensitive indicator of incomplete derivatization [28]. In case of insufficient silylation, the peak of 1,2,4-butanetriol appears splitted and drastically reduced in height.

Mittelbach [29] have prepared the sample by mixing 100–500 μl rapeseed methyl ester (depending upon glycerol content) with 100 μl of 1,4-butanediol standard solution in N,N-dimethylformamide (approximately 0.1 mg 1,4-butanediol/ml) and 600–200 μl of DMF. Finally 200 μl of BSTFA is added to reach the final volume of 1 ml, the mixture was shaken vigorously and 2 μl aliquots were injected in the GC after at least 10 min. It is necessary to use fresh BSTFA reagent to guarantee quantitative derivatization. The GC capillary column used was Fused silica, 60 m × 0.25 mm, 0.25 μm DB 5 (JandW Scientific Inc.) where as Boocock [21] has used the DB-1 fused silica capillary column of dimension 2 m × 0.25 mm i.d. coated with 0.25 μm film of 100% polymethyl siloxane.

Most reports on the use of GC for biodiesel analysis employ flame-ionization detectors (FID), although the use of mass spectrometric detector (MSD) would eliminate any ambiguities about the nature of the eluting materials since mass spectra unique to individual compounds would be obtained [30].

6.2. High performance liquid chromatography method

A general advantage of HPLC compared to GC is that time and reagent consuming derivatization are not necessary, which reduces analysis time. The first literature on HPLC method describes the determination of overall content of mono-, di- and tri-glycerides in fatty acid methyl esters by isocratic liquid chromatography using a density detector. The separation was achieved by coupling a cyano-modified silica column with two GPC columns; chloroform with an ethanol content of 0.6% is used as an eluent. This system allowed for the detection of mono-, di- and tri-glycerides as well as methyl esters as classes of compounds. The system was useful for the study of degree of conversion of the transesterification reaction [31].

HPLC with pulsed amperometric detection (the detection limit is usually 10–100 times lower than for amperometric detection and the detection limit is 1 μg/g) was used to determine the amount of free glycerol in vegetable oil esters. The HPLC-PAD method has proved to be simple, rapid and accurate [32]. The major advantage of this method is its high sensitivity. The simultaneous detection of residual alcohol is also possible with this technique [30].

In an extensive study [33] reverse phase high performance liquid chromatography (RP-HPLC) was used for the determination of compounds occurring during the production of biodiesel from rapeseed oil with various detection methods: UV detection at 205 nm, evaporative light scattering detection (ELSD) and atmospheric pressure chemical ionization mass spectrometry (APCI-MS) in positive ion mode. Two gradient solvent systems were used: one consisting of methanol (A) with 2-propanol-hexane (5:4, v/v) from 100% A to 50:50 A:B—a non-aqueous reverse phase system and the other consisting of water (A), acetonitrile (B) and 5:4 2-propanol–hexane (C) with two linear gradient steps (30% A+70% B in 0 min, 100% B in 10 min, 50% B+50% C in 20 min followed by isocratic elution with 50% B+50% C for last 5 min). The first solvent system was used for rapid quantitative determination of the yield of the transesterification reaction of rapeseed oil with methanol by comparing the sum of the areas of the peaks of methyl esters and triglycerides. The sincerity and linearity of each detection method varied with the individual triglycerides. ELSD and APCI-MS have decreased sensitivity with increasing number of double bonds in the fatty acid methyl esters. However, the sensitivity of UV detection is also different for individual triglycerides. APCI-MS is stated to be the most appropriate detection method for the analysis of rapeseed oil biodiesel.

HPLC method of quantification of mono-, di-, tri-glycerides and esters using UV detection method at the region of 205 nm was studied for methanolysis of rapeseed oils [34]. The glass column 150×3 mm with pre-column 30×3 mm, both packed with C-18, particles with diameter 7 μm and the mobile phase A (acetonitrile:water 80:20), B (acetronitrile), C (hexane:2-propanol 40:50) with 0–2 min—100% A, 2–12 min—change to 100% B, 12–22 min—change to 50% B and 50% C, 22–29 min—change to 100% B, 30–32 min—change to 100% of B, 32–33 min change to initial 100% A.

An HPLC equipped with a refractive index detector was use for the analysis biodiesel produced from soybean oil [35]. The HPLC mobile phase was consisted of acetone and acetonitrile. Mono-, di-, and tri-glycerides were quantified to study the reaction kinetics for transesterification.

6.3. Gel permiation chromatography method

A method for simultaneous analysis of transesterification reaction products-mono-glycerides, diglycerides, triglycerides, glycerol and methyl esters—was developed using gel permeation chromatography coupled with refractive index detector [36]. The mobile phase was HPLC grade tetrahydrofuran at a flow rate of 0.5 mL/min at room temperature and the sample injection size was 10 μL. Sample preparation involves only dilution and neutralization. For analysis, 300 mg of the sample was taken from transesterification reactor and neutralized by adding 5 ml HPLC grade tetrahydrofuran and one drop of 0.6 N HCl. The samples were then kept at 20 °C until analysis. Reproducibility of the method was good: analysis of palm oil transesterification products at different levels of conversion showed a relative standard deviation of 0.27–3.87%. Similarly GPC was used to evaluate the influence of different variables affecting the transesterification of rapeseed oil with anhydrous ethanol and sodium ethoxide as catalyst [37]. GPC has made the quantitation of ethyl esters, mono-, di- and tri-glycerides and glycerol possible.

6.4. ^1H NMR method

The first report on spectroscopic determination of the yield of transesterification reaction utilized ^{1}H NMR depicting its progressing spectrum [38]. The signal due to methylene protons adjacent to the ester group in triglycerides appear at 2.3 ppm and after the reaction the methoxy protons of the methyl esters appear at 3.7 ppm (Fig. 6). These authors have used the areas of the signals of methylene and methoxy protons to monitor the yield of transesterification.

A simple equation is given by the authors:

$$C = 100 \times \left(\frac{2A_{\mathrm{ME}}}{3A_{\mathrm{CH_2}}}\right)$$

where C, percentage conversion of triglycerides to corresponding methyl ester; A_{ME}, integration value of the protons of the methyl esters (the strong singlet); and $A_{\mathrm{CH_2}}$, integration value of the methylene protons.

The factors 2 and 3 derived from the fact that the methylene carbon possesses two protons and the alcohol (methanol derived) carbon has three attached protons.

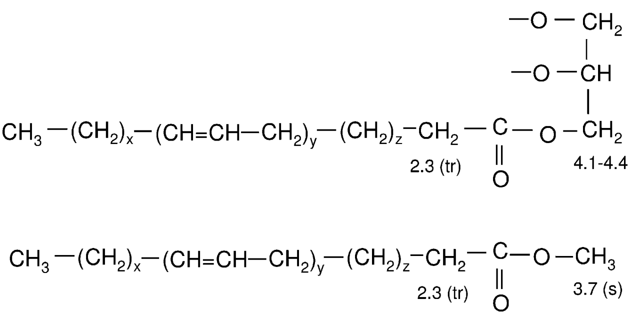


Fig. 6. Assignment of chemical shifts of protons in transesterification reaction.

6.5. NIR spectroscopy

More recently, NIR spectroscopy has been used to monitor the transesterification reaction [40]. The basis for quantitation of the turn over from triglyceride feedstock to methyl ester product is differences in the NIR spectra of these classes of compounds. At 6005 cm^{-1} and 4425–4430 cm^{-1}, the methyl esters display peaks, while triglycerides display only shoulders. Ethyl esters could be distinguished in a similar fashion. Using quantitation software, it is possible to develop a method for quantifying the turnover of triglycerides to methyl esters. The absorption at 6005 cm^{-1} gave better result than the one at 4425 cm^{-1}. The mid range IR spectra of triglycerides and methyl esters of fatty acids are almost identical and offer no possibility for distinguishing.

NIR spectra were obtained with the aid of a fibre-optic probe coupled to the spectrometer, which render their acquisition particularly easy and time-efficient.

While the first NIR paper used a model system to describe monitoring of transesterification and for developing quantitation methods, a second paper applied the method to a transesterification reaction in progress on a 6L scale. Here spectroscopic results were obtained not only by NIR but also by ^{1}H-NMR and NIR [39]. The results of both spectroscopic methods, which can be correlated by simple equations, were in good agreement. Two NMR approaches were used, one being the use of methyl esters protons (peak at 3.6 ppm) and the protons on the carbons next to the glyceryl moiety (α-CH_2; peaks at 2.3 ppm). The second approach was the use of methyl ester protons and the protons of the glyceryl moiety (peaks at 4.1–4.4 ppm) in the triglycerides using the equation:

$$C = 100 \times \frac{5 \times I_{\mathrm{ME}}}{5 \times I_{\mathrm{ME}} + 9 \times I_{\mathrm{TAG}}}$$

where C, percentage conversion of triglycerides to corresponding methyl ester; I_{ME}, integration value of the methyl esters peak; I_{TAG}, integration value of the glyceridic peaks in the triacylglycerol.

7. Fuel properties and specification of biodiesel

Since biodiesel is produced in quite differently scaled plants from vegetable oils of varying origin and quality, it was necessary to install a standardization of fuel quality to guarantee engine performance without any difficulties. Austria was the first country in the world to define and approve the standards for rapeseed oil methyl esters as diesel fuel. As standardization is a prerequisite for successful market introduction and penetration of biodiesel, standards or guidelines for the quality of biodiesel have also been defined in other countries like Germany, Italy, France, the Czech Republic and in the United states.

The parameters, which define the quality of biodiesel, can be divided into two groups. One group contains general parameters, which are also used for mineral oil based fuel, and the other group especially describes the chemical composition and purity of fatty acid alkyl esters [41]. Table 1 contains the general and Table 2 the vegetable oil specific parameters and the corresponding value of fatty acid methyl esters according to standards of above countries.

Table 1
General parameters of the quality of biodiesel

Parameters	Austria (ON)	Czech republic (CSN)	France (journal official)	Germany (DIN)	Italy (UNI)	USA (ASTM)
Density at 15 °C g/cm^3	0.85–0.89	0.87–0.89	0.87–0.89	0.875–0.89	0.86–0.90	–
Viscosity at 40 mm^2/s	3.5–5.0	3.5–5.0	3.5–5.0	3.5–5.0	3.5–5.0	1.9–6.0
Flash point (°C)	100	110	100	110	100	130
CFPP (°C)	0/−5	−5	–	0–10/−20	–	–
Pour point (°C)	–	–	−10	–	0/−5	–
Cetane number	≥49	≥48	≥49	≥49	–	≥47
Neutralization number (mgKOH/g)	≤0.8	≤0.5	≤0.5	≤0.5	≤0.5	≤0.8
Conradson carbon residue (%)	0.05	0.05	–	0.05	–	0.05

Table 2
Vegetable oil specific parameters for the quality of biodiesel

Parameters	Austria (ON)	Czech republic (CSN)	France (journal official)	Germany (DIN)	Italy (UNI)	USA (ASTM)
Methanol/ ethanol (% mass)	≤0.2	–	≤0.1	≤0.3	≤0.2	–
Ester content (% mass)	–	–	≥96.5	–	≥98	–
Monoglyceride (% mass)	–	–	≤0.8	≤0.8	≤0.8	–
Diglyceride (% mass)	–	–	≤0.2	≤0.4	≤0.2	–
Triglyceride (% mass)	–	–	≤0.2	≤0.4	≤0.1	–
Free glycerol (% mass)	≤0.02	≤0.02	≤0.02	≤0.02	≤0.05	≤0.02
Total glycerol (% mass)	≤0.24	≤0.24	≤0.25	≤0.25	–	≤0.24
Iodine number	≤120	–	≤115	≤115	–	–

Among the general parameters for biodiesel, the viscosity controls the characteristics of the injection from the diesel injector. The viscosity of fatty acid methyl esters can go very high levels and hence it is important to control it within an acceptable level to avoid negative impacts on fuel injector system performance. Therefore, the viscosity specifications proposed are nearly same as that of the diesel fuel.

Flash point of a fuel is the temperature at which it will ignite when exposed to a flame or spark. The flash point of biodiesel is higher than the petrodiesel, which is safe for transport purpose.

Cold filter plugging point (CFPP) of a fuel reflects its cold weather performance. At low operating temperature fuel may thicken and might not flow properly affecting the performance of fuel lines, fuel pumps and injectors. CFPP defines the fuels limit of filterability, having a better correlation than cloud point for biodiesel as well as petrodiesel. Normally either pour point or CFFP are specified. Pour point is the lowest temperature at which the oil specimen can still be moved. French and Italian biodiesel specifications specify pour point where as others specify CFPP.

Cetane number is indicative of its ignition characteristics. The cetane number measures how easily ignition occurs and the smoothness of combustion. Higher the cetane number better it is in its ignition properties. Cetane number affects a number of engine performance parameters like combustion, stability, driveability, white smoke, noise and emissions of CO and HC. Biodiesel has higher cetane number than conventional diesel fuel, which results in higher combustion efficiency.

Neutralization number is specified to ensure proper ageing properties of the fuel and/or a good manufacturing process. It reflects the presence of free fatty acids or acids used in manufacture of biodiesel and also the degradation of biodiesel due to thermal effects.

Carbon residue of the fuel is indicative of carbon depositing tendencies of the fuel. Conradsons Carbon Residue for biodiesel is more important than that in diesel fuel because it shows a high correlation with presence of free fatty acids, glycerides, soaps, polymers, higher unsaturated fatty acids and inorganic impurities.

The presence of high level of alcohol in biodiesel cause accelerated deterioration of natural rubber seals and gaskets. Therefore control of alcohol content is required. Biodiesel fuel is mainly consists of fatty acid alkyl esters and its quantities are specified according to the specifications of various countries. The presence of mono- di- and triglycerides cause engine problems like fuel filter plugging affecting the fuel properties and are specified in most of the biodiesel standards.

7.1. Biodiesel storage stability

One of the main criteria for the quality of biodiesel is the storage stability. Vegetable oil derivatives especially tend to deteriorate owing to hydrolytic and oxidative reactions. Their degree of unsaturation makes them susceptible to thermal and/or oxidative polymerization, which may lead to the formation of insoluble products that cause problems within the fuel system, especially in the injection pump. The storage stability of biodiesel prepared from rapeseed oil was investigated by Mittelbach and Gangl [42]. The neutralization number and peroxide number of the biodiesel were observed on long-term storage.

Bondioli et al. [43] have presented the results obtained from a long-term storage study using eleven different biodiesel samples. These samples prepared from several feedstocks using different manufacturing technologies, some containing an antioxidant additive, were stored in 200 L drums. These were periodically monitored during the complete storage period by analysis of fifteen different properties.

Several properties do not show any significant change during storage, while others such as viscosity, peroxide value and more dramatically, Rancimat Induction Period demonstrated changes related to the nature of the starting product.

A parallel test, carried out in simulated wrong storage conditions lead to some strong changes in biodiesel composition and can be used as a guide for devising biodiesel production set-up, storage and distribution chain.

8. Conclusion

Biodiesel has become more attractive to replace petroleum fuel. As per the reported literature, most of the transesterification studies have been done on edible oils like rapeseed, soybean, sunflower, canola etc. by using methanol and NaOH/KOH as catalyst. There are very few studies reported on non-edible oils, which are produced in the wastelands/wild species like *Pongamia pinnata, Jatropha curcas, Simarouba glauca*, etc. The alkaline catalyzed transesterification is the promising area of research for production of biodiesel but for high free fatty acid content non-edible oils, it is one of the main constrains. The use of heterogeneous catalysts is a new area in the development of suitable catalysts in the transesterification of high FFA oil. Ethanolysis is one of the important aspects for biodiesel production as ethanol is derived from renewable biomass. But, the yield of fatty acid ethyl esters is less compared to methyl esters as well as separation of glycerol is the main constrains in the process of ethanolysis. This process can be further improved.

The quality of biodiesel is most important for engine part of view and various standards have been specified to check the quality. As per the analytical method reported in literature, high performance liquid chromatography method is suitable to analyze the reaction intermediates and products of transesterification reaction.

References

[1] Srivastava A, Prasad R. Triglycerides-based diesel fuels. Renew Sustain Energy Rev 2000;4:111–33.
[2] Shay EG. Diesel fuel from vegetable oil: status and opportunities. Biomass Bioenergy 1993;4(4):227–42.
[3] Freedman B, Butterfield RO, Pryde EH. Transesterification kinetics of soybean oil. J Am Oil Chem Soc 1986;63(10):1375–80.
[4] Ma F, Hanna MA. Biodiesel production: a review. Bioresour Technol 1999;70:1–15.
[5] Schuchardt U, Sercheli R, Vargas RM. Transesterification of vegetable oils: a review. J Braz Chem Soc 1998;9(1):199–210.
[6] Dorado MP, Ballesteros E, Almeida JA, Schellet C, Lohrlein HP, Krause R. An alkali-catalyzed transesterification process for high free fatty acid oils. Trans ASAE 2002;45(3):525–9.

[7] Ma F, Clements LD, Hanna MA. The effect of catalyst, free fatty acids, and water on transesterification of beef tallow. Trans ASAE 1998;41(5):1261–4.
[8] Freedman B, Pryde EH, Mounts TL. Variables affecting the yield of fatty esters from trasnsesterified vegetable oils. J Am Oil Chem Soc 1984;61(10):1638–43.
[9] Canakci M, Gerpen JV. Biodiesel production from oils and fats with high free fatty acid. Trans ASAE 2001; 44(6):1429–36.
[10] Turck R. Method for producing fatty acid esters of monovalent alkyl alcohols and use thereof. USP 0156305 2002.
[11] Ahn E, Mittelbach M, Marr R. A low waste process for the production of biodiesel. Sep Sci Technol 1995; 30(7–9):2021–33.
[12] Tomasevic AV, Marinkovic SS. Methanolysis of used frying oils. Fuel Process Technol 2003;81:1–6.
[13] Gryglewicz S. Rapeseed oil methyl esters preparation using heterogeneous catalysts. Bioresour Technol 1999;70:249–53.
[14] Mohamad IAW, Ali OA. Evaluation of the transesterification of waste palm oil into biodiesel. Bioresour Technol 2002;85:25–256.
[15] Fuduka H, Kondo A, Noda H. Biodiesel fuel production by transesterification of oils. J Biosci Bioeng 2001; 92(5):405–16.
[16] Enciner JM, Gonzalez JF, Rodriguez JJ, Tajedor A. Biodiesels fuel from vegetable oils: transesterification of *Cynara cardunculus* L. oils with ethanol. Energy Fuels 2002;16:443–50.
[17] Zhou W, Konar SK, Boocock DGV. Ethyl esters from the single-phase base-catalyzed ethanolysis of vegetable oils. J Am Oil Chem Soc 2003;80(4):367–71.
[18] Ma F, Clements LD, Hanna MA. The effect of mixing on transesterification of beef tallow. Bioresour Technol 1999;69:289–93.
[19] Boocock DGB, Konar SK, Mao V, Sidi H. Fast one-phase oil-rich processes for the preparation of vegetable oil methyl esters. Biomass Bioenergy 1996;(11):43–50.
[20] Boocock DGB, Konar SK, Mao V, Lee C, Bulugan S. Fast formation of high-purity methyl esters from vegetable oils. J Am Oil Chem Soc 1998;75(9):1167–72.
[21] Boocock DGB. Single phase process for production of fatty acid methyl esters from mixtures of triglycerides and fatty acids. WO 0112581 2001.
[22] Boocock DGB. Process of producing lower alkyl fatty acid esters. CA 0112581 1996.
[23] Krisnangkura K, Simamaharnnop R. Continuous transmethylation of palm oil in an organic solvent. J Am Oil Chem Soc 1992;69(2):166–9.
[24] Jackson MA, King JW. Methanolysis of seed oils in supercritical carbon dioxide. J Am Oil Chem Soc 1996; 73(3):353–6.
[25] Saka S, Kusdiana D. Biodiesel fuel from rapeseed oil as prepared in supercritical methanol. Fuel 2001;80: 225–31.
[26] Kudsiana D, Saka S. Methyl esterification of free fatty acids of rapeseed oil as treated in supercritical methanol. J Chem Eng Jpn 2001;34(3):373–87.
[27] Freedman B, Pryde EH, Kwolek WH. Thin layer chromatography/flame ionization analysis of transesterified vegetable oils. J Am Oil Chem Soc 1984;61(7):1215–20.
[28] Plank C, Lorbeer E. Simultaneous determination of glycerol, mono-, di-, and triglycerides in vegetable oil methyl esters by capillary gas chromatography. J Chromatogr A 1995;697:461–8.
[29] Mittelbach M. Diesel fuel from vegetable oils, V: gas chromatographic determination of free glycerol in transesterified vegetable oils. Chromatographia 1993;37(11–12):623–6.
[30] Knothe G. Analytical methods used in the production and fuel quality assessment of biodiesel. Trans ASAE 2001;44(2):193–200.
[31] Trathnigg B, Mittelbach M. Analysis of triglyceride methanolysis mixtures using isocratic HPLC with density detection. J Liq Chromatogr 1990;13(1):95–105.
[32] Lozano P, Chirat N, Graille J, Pioch D. Measurement of free glycerol in biofuels. Fresenius J Anal Chem 1996;354:319–22.
[33] Holcapek M, Jandera P, Fischer J, Prokes B. Analytical monitoring of the production of biofuel by high performance liquid chromatography with varius detection methods. J Chromatogr A 1999;858:13–31.

[34] Komers K, Stloukal R, Machek J, Skopal F. Biodiesel from rapeseed oil, methanol and KOH. 3. Analysis of composition of actual mixture. Eur J Lipid Sci Technol 2001;103(6):363–71.
[35] Noureddini H, Zhu D. Kinetics of transesterification of soybean oil. J Am Oil Chem Soc 1997;74(11): 1457–63.
[36] Darnoko D, Cheryan M, Perkins EG. Analysis of vegetable oil transesterification products by gel permiation chromatography. J Liq Chrom Rel Technol 2000;23(15):2327–35.
[37] Fillieres R, Mlayah BB, Delmas M. Ethanolysis of rapeseed oil: quantitation of ethyl esters, mono-, di-, and triglycerides and glycerol by high performance size-exclusion chromatography. J Am Oil Chem Soc 1995; 72(4):427–32.
[38] Gelbard G, Bres O, Vargas RM, Vielfaure F, Schuchardt UF. ^{1}H nuclear magnetic resonance determination of the yield of the transesterification of rapeseed oil with methanol. J Am Oil Chem Soc 1995;72(10): 1239–41.
[39] Knothe G. Monitoring a processing transesterification reaction by fibre-optic color near infrared spectroscopy with correlation to ^{1}H nuclear magnetic resonance. J Am Oil Chem Soc 2000;77(5):489–93.
[40] Knothe G. Rapid monitoring of transesterification and accessing biodiesel fuel quality by near-infrared spectroscopy using a fibre optic probe. J Am Oil Chem Soc 1999;76(7):795–800.
[41] Mittelbach M. Diesel fuel derived from vegetable oils, VI: Specifications and quality control of biodiesel. Bioresour Technol 1996;27(5):435–7.
[42] Mittelbach M, Gangl S. Long term storage stability of biodiesel made from rapeseed and used frying oil. J Am Oil Chem Soc 2001;78(6):573–7.
[43] Bondioli P, Gasparoli A, Bella LD, Tagliabue S, Toso G. Biodiesel stability under commercial storage conditions over one year. Eur J Lipid Soc Technol 2003;105(12):735–41.